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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Novel Boronic Chalcone Derivatives & Uses Thereof

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Application Date Sheet. See 37 CFR 1.76

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Respectfully submitted,

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U.S. Provisional Patent Application

JHU Ref. No.: JHU-4085

Novel Boronic Chalcone Derivatives and Uses Thereof

Inventor: Saeed R. Khan

NOVEL BORONIC CHALCONE DERIVATIVES AND USES THEREOF

RELATED APPLICATIONS

Application No. 60/388,255 filed June 13, 2002, and U.S. Provisional Patent Application No. 60/444,429 filed February 3, 2003, both of which are incorporated herein in their entirety by this reference.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel boronic chalcone compounds and uses thereof. The compounds of this invention are particularly useful for the treatment of tumors and cancers.

Description of the State of the Art

Throughout this application, various publications are referenced by author and date. Full citations for these publications may be found listed at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference in their entireties into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of this invention described and claimed herein.

Breast cancer is expected to account for 203,500 new cancer cases and 39,600 deaths in 2002 (Jemal, A. et al., *CA Cancer J. Clin.*, 2002, 52, 23-47). Although major advances have been made in early detection, prevention, and treatment, the need for more effective therapy in the fight against late stage breast cancer continues. Currently there is no curative treatment for women with metastatic breast cancer once they have failed adjuvant therapies. New and effective cytotoxic agents with novel mechanisms of action are therefore urgently needed for the treatment of women with metastatic breast cancer. Hormone and chemotherapy, while of substantial palliative benefit, has had little impact on overall survival and the

mortality rate from metastatic breast cancer. At the present time standard treatments for metastatic breast cancer include paclitaxel in combination with vinca alkaloids, etoposide, and other regimens that include agents such as anthacyclines, alkylating agents, antimetabolites, tamoxifen, and aromatase inhibitors (Chabner BA, Collins JM, Cancer chemotherapy principal and practice, pp 9-13, and 40-85. B. Lippincott Company, Philadelphia, 1990). The ultimate conclusion of these numerous studies over the last 50 years is that although these therapies provides significant palliative effect in the majority of with metastatic breast patients, it is likely to be curative. Although major advances have been made in early detection, prevention, and treatment of early disease, the need for more effective therapy in the fight against late stage breast cancer continues.

Recently the mouse double minute 2 (MDM2) oncogene has been suggested as a target for breast cancer therapy (Juven-Gershon, T. and Oren, M. *Mol. Med.*, 1999, 5, 71-83; Momand, J. et al., *Nucleic Acids Res.*, 1998, *26*, 3453-3459). MDM2 is amplified or overexpressed in human breast cancer, and MDM2 levels are associated with poor prognosis of human breast cancer. The oncoprotein MDM2 inhibits the tumor suppressor protein p53 by binding to the p53 transactivation domain. The p53 gene is inactivated in human cancer either by mutations or by binding to oncogenic proteins such as MDM2 (Lane, D. P. and Hall, P. A., *Trends Biochem. Sci,*. 1997, 22, 372-374; Oliner, J. D. et al., *Nature,* 1992, *358*, 80-83; Lozano, G.; Montes de Oca Luna, R., *Biochim. Biophys. Acta*, 1998, *1377*, M55-M59; Wang, H. et al., *Clinical Cancer Res.*, 2001, *7*, 3613-3624). In breast tumors, over expression of MDM2 inactivates an otherwise intact p53, disabling the genome integrity checkpoint and allowing cell cycle progression of defective cells (Boyd, M. T. et al., *J. Biol. Chem.*, 2000, *275*, 31883-31890). Studies comparing MDM2 overexpression and p53 mutation concluded that these are mutually exclusive events, supporting the notion that the primary impact of MDM2 amplification in cancer cells is the inactivation of the endogenous wild-type p53 (Wang et al., *supra*). It has been shown recently that a peptide homologue of p53 is

sufficient to induce p53-dependent death of cells overexpressing MDM2 (Wasylyk, C., et al., *Oncogene*, 1999, *18*, 1921-1934). This result provides clear evidence that disruption of the p53/MDM2 complex might be effective in cancer therapy. It has been shown that MDM2 additionally has a role in tumor growth p53-independent mechanisms (Baker, S. J., et al., *Science*, 1990, *249*, 912-915; Diller, L., et al., *Mol. Cell. Biol.*, 1990, *10*, 5772-5781; Fakharzadeh, S. S., et al., *EMBO J.*, 1991, *10*, 1565; Lundgren, K.; Montes de Oca Luna, R., et al., *Genes Dev.*, 1997, *11*, 714-725; Zhang, R. and Wang, H., *Curr. Pharm. Des.*, 2000, *6*, 393-416; Chabner, B. A. and Collins, J. M., *Cancer chemotherapy principal and practice*; Lippincott Williams & Wilkins Publishers: Philadelphia, 1990; pp 9-13 and 40-85B).

Chalcones are a class of anticancer agents that have shown promising therapeutic efficacy for the management of human cancers. Chalcones, considered as the precursor of flavonoids and isoflavonoids, are abundant in edible plants. Chemically they comprise open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system. For example, chalcones have been observed to inhibit the proliferation of both established and primary ovarian cancer cells (De Vincenzo, R., et al., *Anticancer Drug Des.*, 1995, *10*, 481-490). *In vivo*, chalcones have been demonstrated to be effective as antitumor agents in skin carcinogenesis (Statomi, Y., *Int. J. Cancer*, 1993, *55*, 506-514; Yamamoto, S. et al., *Carcinogenesis*, 1991, *12*, 317-323) and chemopreventive agents in several experimental models (Makita, H., et al., *Cancer Res.*, 1996, *56*, 4904-4909; Rui, H., *J. Cell. Biochem.*, 1997, *67*, 7-11; Wattenberg, L. W., et al., *Cancer Lett.*, 1994, *83*, 165-169). Recent studies have shown that these chalcones induce apoptosis in variety of cell types including breast cancers (Claude-Alain, C., et al., *Anticancer Res.*, 2001, *21*, 3949-3956; WO 01/117988; WO 96/19209; U.S. Patent No. 5,808,137; Maggiolini, M., et al., *J. Steroid Biochem. Mol. Biol.*, 2002, *82*, 315-322; Stoll, R., et al., *Biochemistry*, 2001, *40*, 336-344; DiCesare, N. and Lakowicz, J. R., *Tetrahedron. Lett.*, 2002, *43*, 2615-2618). Biochemical experiments have shown that these compounds could disrupt the

MDM2/p53 protein complex, releasing p53 from both the p53/MDM2 and DNA-bound p53/MDM2 complexes (Stoll et al., *supra*).

Carboxylic chalcones have shown promising therapeutic efficacy for the management of human cancers (Daskiewicz, J. B., et al., *Tetrahedron Lett.* 1999, *40*, 7095-7098; Devincenzo, R., et al., *Anti-Cancer Drug Des.* 1995, *10*, 481-490). Previous studies (Stoll et al., *supra*; Kussie, P. H., et al., *Science*, 1996, *274*, 948-953) on the binding modes of carboxylic acid analogs of chalcones with MDM2 revealed that the carboxylic acid group could be placed near the base of lysine51 (K51), which is found in a salt bridge interaction with glutamic acid 25 (E25). It was presumed that the acid group of the chalcone forms a salt bridge with K51 and simultaneously breaks the salt bridge with E25 of the MDM2. However, carboxylic acid analogs of chalcone reported in the literature (Stoll et al., *supra*) are equally toxic to both normal and malignant breast epithelial cells. The toxicity to normal breast cells may be due to MDM2/p53 independent mechanisms. Therefore, a chalcone derivative that could strongly and irreversibly bind to and disrupt MDM2 protein complexes may be selectively toxic to MDM2 overexpressing breast cancer cells.

Boronic acids are Lewis acids and isosteres of carboxylic acid. The pKa's of boronic acids are about 9-10, and therefore at physiological pH boronic acids remain unionized (Tongcharoensirikul, P., et al., *Abstracts of Papers*, 222nd ACS national meeting, Chicago, IL, August 26-30; American chemical society, Washington, DC, 2001; MEDI-224). Thus, a coordinate covalent bond (boron-nitrogen) can be formed between a electron deficient boronic acid moiety and electron donating amino group, which may strongly enhance binding of boronic-chalcones with the lysine 51 of MDM2 at neutral pH when compared to the corresponding carboxylic acid analog of chalcones.

Boronic chalcone analogs have been previously described. These compounds have been used as fluorescent probes that may be useful for detection of fluorides (DiCesare, N. and Lakowicz, J. R., *supra*) and

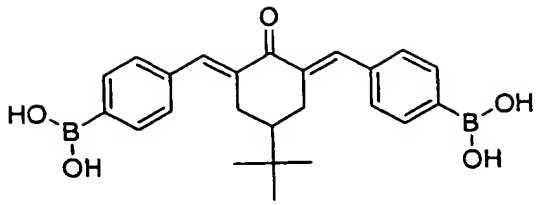
saccharides such as glucose that may be applicable to the design of biosensors for diabetes (DiCesare, N. and Lakowicz, J. R., *supra*). However, prior to this invention no investigations into the anticancer activity of boronic-chalcones on different cancer cell lines have been reported.

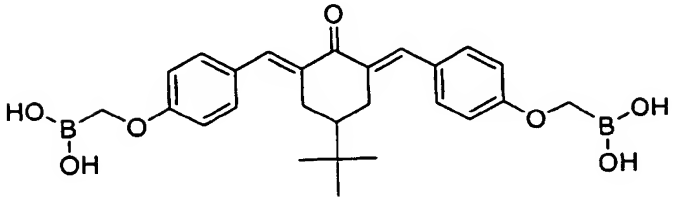
SUMMARY OF THE INVENTION

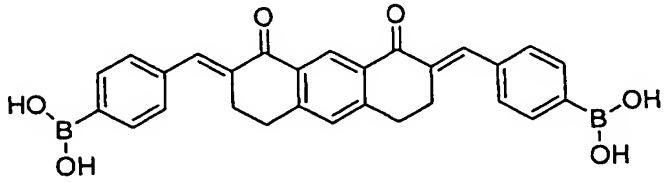
Surprisingly, it has now been found that certain novel chalcones derivatives, in particular boronic chalcone derivatives, possess antiproliferative activity on cancer cells at micromolar concentrations. Accordingly, this invention provides the design and synthesis of novel boronic chalcone derivatives, and pharmaceutical compositions containing these compounds. Several the compounds described herein were observed to have high activity in the breast cancer cell lines tested and has been shown to be 6-9 fold less toxic to normal MCF-12A cell lines compared to normal breast epithelial cell lines. The novel boronic chalcone analogs disclosed herein should overcome the limiting lack of specificity of carboxylic acid analogs of chalcones.

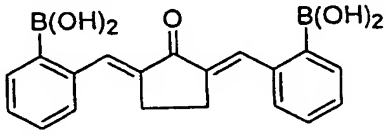
The present invention further investigates the potential value of MDM2 as a drug target for breast cancer therapy. For example, a chalcone derivative of this invention that inhibits MDM2 expression or binds to and disrupts the MDM2 protein complex may be a useful compound for the treatment of breast cancer. While not wishing to be bound by any theory, it is believed that the boronic acid analog might form a stronger salt bridge with K51 of MDM2 than the corresponding carboxylic acid analogs of chalcones and will selectively inhibit growth of breast cancer cells. Accordingly, a set of boronic acid-chalcone derivatives were designed and tested their ability to selectively kill breast cancer over normal breast epithelial cells.

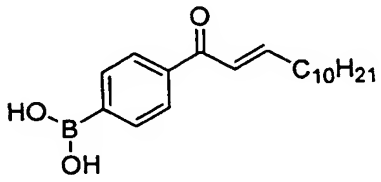
In general, one embodiment of the invention relates to boronic-chalcone compounds of the general Formula I:

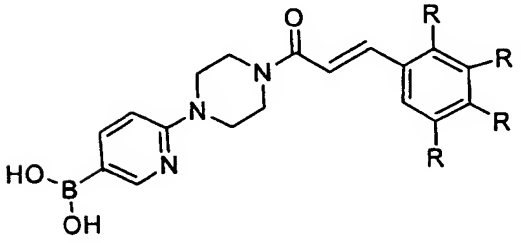




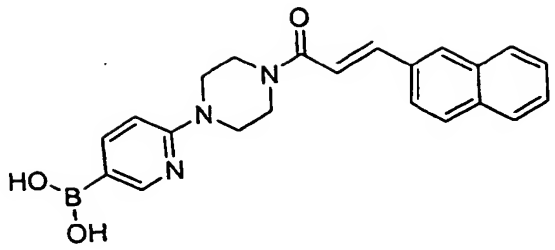








R= halogen (e.g., fluoro, chloro, bromo, and iodo groups), alkyl, heteroalkyl, alkoxy, heteroalkoxy, hydroxamic acid



All publications, patents and patent applications disclosed herein are incorporated into this application by reference in their entirety.

For example: "Sambrook *et al.*, Molecular Cloning, A Laboratory Manual (volumes I-III) 1989, Cold Spring Harbor Laboratory Press, USA", "Harlowe and Lane, Antibodies a Laboratory Manual 1988 and 1998, Cold Spring Harbor Laboratory Press, USA" and "Ausubel *et al.*, Current Protocols 2001, John Wiley and sons, Inc." provide sections describing methodology for antibody generation and purification, diagnostic platforms, cloning procedures, etc. that may be used in the practice of the instant invention.

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